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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
Nasmyth *et al.*	Art Unit: 1655
Appl. No. 09/308,109	Examiner: Lu, F.
102(e) Date: June 2, 1999	Atty. Docket: 0652.1880000/EKS/VSS
For: **Methods for Producing the Anaphase Promoting Complex**	

Amendment And Reply Under 37 C.F.R. § 1.111

Commissioner for Patents
Washington, D.C. 20231

Sir:

In reply to the Office Action dated **July 17, 2001,** (PTO Prosecution File Wrapper Paper No. 15), Applicants submit the following Amendment and Remarks. This Amendment is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks and arguments. 37 C.F.R. § 1.111 and MPEP 714; and

(C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned

under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Claims:

Please cancel claims 11-13 without prejudice or disclaimer.

Please add the following claims:

14. (new) A method for identifying substances that inhibit rapidly proliferating cells by interfering with the cells' entry into the subsequent cell cycle comprising:

a) incubating Anaphase Promoting Complex (APC), which is capable of ubiquitinating cyclin B, in the presence of a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2), ubiquitin, ATP and an APC substrate, with a test substance; and

b) determining the effect of said test substance on the APC's ability to ubiquitinate said substrate, wherein if the amount of ubiquitination of said substrate is less than in reactions containing

no test substance, then said test substance inhibits APC-dependent cell cycle progression.

15. (new) The method of claim 14, wherein said APC is yeast APC.

16. (new) The method of claim 14, wherein said APC is human APC.

17. (new) The method of claim 14, wherein said APC comprises one or more polypeptides selected from the group APC1, CDC16, CDC23, CDC26, CDC27, APC2, APC5, APC4, APC9, and APC11, or homologues thereof.

18. (new) The method of claim 14, wherein the said APC is expressed in a Baculovirus expression system.

19. (new) The method of claim 14, wherein said APC substrate is cyclin B.

20. (new) The method of claim 14, wherein said substrate is of human origin.

21. (new) The method of claim 20, wherein said APC substrate is human cyclin B.

22. (new) A method for identifying substances that inhibit rapidly proliferating cells by interfering with the cells' entry into the subsequent cell cycle comprising:

a) identifying novel subunits of the Anaphase Promoting Complex (APC) by

i) replacing in a cell selected from cells from the budding yeast *Saccharomyces cerevisiae* or from human cells one or more endogenous genes encoding a known APC subunit with epitope-tagged versions of said genes or transforming the cell with a vector containing the corresponding epitope-tagged cDNA(s),

ii) growing cells obtained in i) and preparing a protein extract,

iii) isolating said APC by contacting the protein extract obtained in ii) with an antibody directed against the epitope-tag,

iv) isolating and purifying the antibody-bound protein(s),

v) determining the sequence of the protein(s), and optionally

vi) identifying, in the case that the proteins obtained are yeast proteins, the human subunit(s) by comparing the sequence(s) of the yeast protein(s) and/or the DNA sequence encoding those proteins with published human sequences;

b) producing human or *Saccharomyces cerevisiae* APC containing said APC subunits identified in a) by

i) expressing cDNAs encoding APC subunits,

ii) isolating and purifying said subunits and allowing them to assemble to form a functional APC;

c) incubating said APC obtained in b), in the presence of a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2), ubiquitin, ATP and an APC substrate, with a test substance; and

d) determining the effect of said test substance on the APC's ability to ubiquitinate the substrate, wherein in if the amount of ubiquitination of said substrate is less than in reactions containing no test substance, then said test substance inhibits APC-dependent cell cycle progression.

23. (new) The method of claim 22, wherein said cell is a yeast cell.

24. (new) The method of claim 22, wherein said cell of is a human cell.

25. (new) The method of claim 22, wherein said gene(s) are selected from the group comprising *APC1, CDC16, CDC23, CDC26, CDC27, APC2, APC5, APC4, APC9,* and *APC11,* or homologues thereof.

26. (new) The method of claim 22, wherein said cDNAs in are expressed in a Baculovirus expression system.

27. (new) The method of claim 22, wherein said APC and said substrate of step are of human origin.

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28. (new) The method of claim 22, wherein said APC substrate is cyclin B.

29. (new) The method of claim 28, wherein said APC substrate is human cyclin B.

Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 14-29 will be pending in the application, with claims 14 and 24 being the independent claims. Claims 1-6 are withdrawn from consideration. Claims 11-13 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 14-29 are sought to be added. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Support for this amendment can be found throughout the specification, in particular in the Examples. Specifically, support for claims 14 and 22 (and their dependents) can be found, *inter alia*, on page 7, second full paragraph, through page 8, first full paragraph, of the specification. Support for claim 14 can additionally be found on page 6, sixth paragraph of the specification. Further support for a) and b) of claim 22 can be found on page 2, second paragraph, page 4, first paragraph, in Examples 1-7, with particular support found in Example 2b and Example 4a-b. Further support for claims 15, 16, 23, and 24 can be found on page 7, first and second paragraphs. Support for claims 17 and 25 can be found in Examples 3, 5, 9, and 12. Additional support for claim 19 can be found in Example 1.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 11-13 were rejected under 35 U.S.C. § 112, first paragraph as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. Specifically, the Examiner alleged that undue experimentation would be required to use the screening assay in the manner claimed by the Applicants due to a supposed lack of direction or guidance in the specification on how reconstitute a recombinant APC complex and use any kind of substrate. Applicants respectfully traverse this rejection as it may apply to the amended claims.

Applicants have clearly shown the reconstitution of recombinant APC in yeast, as described in the specification on page 9, third paragraph, second and third sentences, and in detail in the working Example 2, beginning on page 18. To begin, one or more APC subunits were modified and expressed, then the complex was extracted and incubated with a cyclin B substrate, either Clb2p or Clb3p. Further, APC comprising various myc- or hemagglutinin- tagged subunits were shown to function normally in yeast cells, as described in Example 10, page 32, first full paragraph, indicating that the recombinant APC was functional. Applicants note that the nonfunctional APC characterized by these experiments contained subunits that were "knocked out," or rendered inactive, in addition to being tagged. Applicants therefore respectfully submit that a functional recombinant APC has been achieved and that the specification provides sufficient guidance for the preparation of such for the assay of the claimed invention.

Although extracellular reconstitution of recombinant APC is not required for the practice of this invention, it is encompassed by it. The Examiner agrees in general that

certain protein expression systems, such as baculovirus or *Pichia pastoris*, are well known protein expression systems, but contends that because additional APC subunits may be discovered in the future, that reconstitution of APC is presently impractical. Applicants agree that additional polypeptides that associate with APC that are important for understanding the function and regulation of APC in a living cell may be found, but assert that every possible subunit may not be required for the simple assay of the invention. The claims as amended are directed to an APC capable of ubiquitinating a cyclin B, which has been demonstrated in the specification as discussed *supra*. It would be a matter of routine for one skilled in the art to express the subunits already described in the specification or to be discovered using the method of the claimed invention, assemble them *in vitro*, and assay which complexes have this ubiquitination capability. On page 604, halfway through the first paragraph, through page 605, Page *et al.* teaches that the reconstitution of SCF-mediated Sic1p ubiquitination from baculovirus-generated polypeptides provides additional guidance for the reconstitution of APC (Page, A.M. and Hieter, P., *Annu Rev Biochem 68*:583-609 (1999)). The extensive characterization of both human and yeast APC subunit found in the working examples, *inter alia*, of the specification provides guidance for the routine optimization of the method for producing a functional APC *in vitro*. The Examiner is respectfully reminded that "[t]he test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." (*In re Wands*, as quoted in MPEP § 2164.06). Since the methods to produce *in vitro* and identify APC capable of ubiquitinating a cyclin B using the guidance of the specification

require only routine molecular biology, Applicants respectfully submit that such experimentation is not undue.

The substrate

The Examiner alleged that the it is not known whether any kind of substrate that can be ubiquitinated by APC is involved in the initiation of a new cell cycle. APC is known to ubiquitinate a number of substrates important for cell cycle regulation (for a partial list, see Table 1 of Peters, J.M., *Exp Cell Res 248*:339-340 (1999)). Whether the ubiquitination of a particular substrate is required for sister chromatid separation, as questioned by the Examiner on page 4 of the outstanding Office Action, is immaterial, as sister chromatid separation is not the only factor in initiating a new cell cycle. Separation of sister chromatids may not be dependent on the ubiquitination of a particular substrate, though the ubiquitination of that substrate is required for cell cycle progression by affecting other elements of the process. Applicants have clearly shown that the ubiquitination of Clb2p is required for anaphase progression, and that cells in which these polypeptides are not ubiquitinated have an arrested cell cycle. Applicants' work and others as indicated *supra* and in the specification provide other substrates that may be suitable for use in the assay of the claimed invention as the APC-dependent ubiquitination of these substrates is required for cell cycle progression, whether that substrate be involved in sister chromatid separation or another element in the process. Further, the identification of additional substrates suitable for use in the assay of the invention would be routine using the ubiquitination assay and cell cycle progression assays described in the specification and summarized in the discussion *supra*.

Applicants submit that the ubiquitination of substrates by APC is clearly involved in cell cycle progression, and that the use of these substrates in the assay of the claimed invention is fully enabled. Applicants therefore respectfully request that the rejection be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 11-13 have been rejected under 35 U.S.C. § 112, second, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner alleged that the claims are vague and indefinite for failing to include a positive and/or negative control, and for the phrase "recombinant APC." The claims have been amended to recite a positive control without the test substance and to include a function of APC, specifically the ability to ubiquitinate a cyclin B, as described in the specification on page 6, sixth paragraph. Therefore, Applicants respectfully request that the rejection be withdrawn.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will

expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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